

THE DEVELOPMENT AND REPLACEMENT OF PELAGE HAIRS IN THE BANDICOOT *PERAMELES NASUTA* GEOFFROY (MARSUPIALIA : PERAMELIDAE)

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Summary

A histological study has been made of the development and replacement of pelage hairs in the bandicoot, *Perameles nasuta* Geoffroy. Skin samples were taken from the mid-lateral region of the trunks of 18 specimens from eight different litters at estimated ages ranging from birth to 526 days. Six specimens were sampled more than once.

At birth pelage hair follicles are absent, and the first follicles appear in the mid-lateral region about 11 days after birth. The first hairs emerge in this region at about the 40th day, and at 48 days all, or most, of the follicles of the first hair cycle have emerging hairs.

The follicles which develop during this first hair cycle are arranged in groups. Within each group the following follicles may be distinguished: a single original central primary follicle, a pair of original lateral primary follicles, and a number of original secondary follicles. All these original follicle types arise directly from the epidermis and open independently on the skin surface; they are to be distinguished from the derived follicles arising late in the first cycle and in succeeding cycles. Derived follicles develop by branching from existing follicles and share a common orifice with their parent follicle.

The special features in the development of the different types of hair follicles of the first hair cycle are described, and their rates of development are compared.

Before the end of the first hair cycle the derived follicles begin to arise by branching from all follicles, except the central primary of each group, a method of follicle replacement which apparently has not been previously described in mammals.

As successive hair cycles occur, further follicles arise by branching from earlier-formed lateral primary and secondary follicles, and from a few original central primary follicles. This results in the formation of bundles of closely associated follicles with a common orifice, and an increase in size of the follicle group. New original central primary hairs, which merely replace older hairs of similar type, develop in follicles arising from the bases of resting original central primary follicles; they emerge on the ectal, or grooved, sides of the old hairs. The hairs which develop in the derived central primary follicles are circular in cross section and appear to be similar in type to those of secondary follicles.

I. INTRODUCTION

The bandicoot, *Perameles nasuta* Geoffroy, is a typical marsupial in that the young are born at a rather early stage of development and are reared within a pouch. Because of this the pouch young have proved to be very favourable material for studying the early stages in the development of the hair coat; in eutherian mammals this development occurs *in utero*. *P. nasuta*, on the other hand, is born before the

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pelage hair follicles are formed. Periodic surgical excision of skin samples has made it possible to follow the entire development, and the later history, of the hair follicles in one and the same specimen.

Since the structure and development of the skin and hair in marsupials appears to be fundamentally similar to that of eutherian mammals, the use of the more favourable marsupial material for the study of many of the basic problems associated with the biology of the mammalian integument seems to be fully justified.

The only known study of marsupial hair development seems to be that of Gibbs (1938) on the development of the skin and hair in pouch young of the brush-tailed possum *Trichosurus vulpecula* Kerr. *T. vulpecula* and several other marsupials are exceptional in that practically the whole of the skin is covered by a coat of hairs before the vibrissae emerge (Lyne, unpublished data). Gibbs drew attention to this unique character of hair formation in *T. vulpecula* and suggested that it is probably associated with the development in the pouch. However, Gibbs was not aware that only very few marsupials show this early development of the first pelage hairs; the shedding and replacement of these hairs, which take place at an early age (Lyne, unpublished data), appear to have been completely overlooked.

The grouping of hair follicles in the skin of marsupials has received little special attention in comparative studies of the mammalian coat. Limited studies have been made by de Meijere (1894) on a number of different marsupials, by Sweet (1907) on *Notoryctes typhlops* Stirling, by Gibbs (1938) and Bolliger and Hardy (1944) on *T. vulpecula*, and by Hardy (1947) on 13 Australian marsupials. In Hardy's study no detailed consideration was given to ontogenetic or phylogenetic relationships, or to the relation between the follicle types in the skin and the hair types in the coat. Hardy pointed out, however, that her study of the adult hair follicle group-arrangement made abundantly clear the need for examining the development of follicle groups in the pouch young of marsupials in order to interpret the adult structure.

The presence of two distinct hair types—large grooved hairs and small circular hairs—in the bandicoots *Isodon obesulus* Shaw & Nodder and *Perameles gunnii* Gray, described by Lyne and McMahon (1951), suggested that either of these marsupials, or related species, would be ideal for the study of the relation between hair types and follicle types.

Although de Meijere (1894) briefly reported the branching of hair follicles in monotremes, marsupials, and various eutherian mammals, the origin of bundles of hair follicles appears to have been overlooked by all but a few authors. Tänzer (1926) described the branching of wool follicles in Karakul sheep and Hardy and Lyne (1956b, 1956c) have shown that the bundles of wool follicles in Merino sheep are formed by the branching of follicles which arise directly from the epidermis. The significance of these findings suggested the advisability of further investigation of some of the problems of hair follicle branching.

In many eutherian mammals the pelage hair follicles exhibit cyclic activity in alternating periods of growth and rest. This aspect of hair growth in *P. nasuta*, not previously described in any marsupial, is briefly outlined in this paper. The particular method of follicle replacement, associated with the hair cycles, has not been previously described in mammals.

II. MATERIAL AND METHODS

(a) *Animals*

During the collecting period (1954–55), 45 adult or nearly adult *P. nasuta* were trapped near Sydney, N.S.W., and were maintained in captivity in this Laboratory. Of these, 20 were females and nine were carrying young in the pouch. Although several litters have been successfully reared to maturity, *P. nasuta* has not, to date, been bred in captivity. Specimens of one litter (No. 2) reared in captivity and used in the present investigation are shown in Plate 1: These specimens had their eyes open at 48 days and were first seen out of the pouch 3 days later. The specimens of another litter (No. 6) had their eyes open at about 45 days after birth and were first seen out of the pouch at 55 days.

A total of 26 pouch young was available for the present investigation and of these 11 were females and 11 were males while four were of unknown sex. In addition to the pouch young, two advanced intra-uterine young were obtained from an adult which died after 11 days in captivity.

The pouch young were measured, sexed when possible, and live weights were obtained at intervals after the period of attachment to the teat. Length of the tail, ear, pes, and manus were recorded in a manner similar to that described by Lyne (1951) and two additional measurements, the head length and crown-rump length, were also obtained.

(b) *Estimation of Age*

The age of eight specimens was estimated from the measurements of two newly born young, given by Hill (1897), and repeated observations on four of these were used to determine the regression of age on the six measurements of length described above. A cubic regression line was fitted for each measurement, and, from the six estimates of age so obtained, a mean was obtained by weighting each estimate with the inverse of its error variance. The accuracy of these estimates was satisfactory for ages of 50 days or over. Within the range 0–50 days a cubic regression line for age on head length was used.

(c) *Skin Sampling and Histological Methods*

A total of 53 post-natal skin samples, taken from the mid-lateral region of the trunk (referred to as the midside region), was obtained by biopsy or immediately after death from 18 specimens from eight different litters. Twelve specimens were sampled once at estimated ages ranging from 1 to 40 days, and six specimens were sampled more than once at estimated ages ranging from 4 to 526 days. Full details of the specimens sampled are given in Table 1.

The midside skin samples were taken from either side of the trunk. When a specimen was sampled more than once the right and left sides were sampled alternately. The third and later samples were taken from a position either slightly anterior or posterior to the midside position. To avoid sampling the same position a second time the scar resulting from the previous sampling was tattooed before it disappeared. All the midside skin samples were fixed in Zenker's fluid. One entire

pouch young was fixed in 5 per cent. formalin; the other pouch young and the intra-uterine young were fixed in Bouin's fluid. Most of the skin samples were divided into two or more pieces after clearing in cedar-wood oil. Usually two pieces

TABLE 1
DETAILS OF SPECIMENS SAMPLED*

Litter No. and Estimated Date of Birth	Specimen No.	Sex	Estimated Age (days) from Birth when Sampled
1 30.v.54	3	?	1
	4	♂	4, 11, 18
2 16.vii.54	5†	♀	6, 20, 34, 48, 62, 76, 83, 97, 125, 137
	6†	♂	6, 20, 34, 48, 62, 76, 83, 97, 125, 153, 181, 209, 265, 321, 377
3 14.x.54	8	♀	21
	9	♂	21
	10	♂	21
	11	♂	21
4 25.xi.54	12	♀	13, 20, 27
5 19.xi.54	16	♀	34
	17	♂	34
	18	♂	34
6 18.xii.54	20	♀	40, 45
	22†	♀	54, 61, 75, 110, 166, 222, 463, 526
	23	♂	40
7 3.vii.55	24‡	?	2
8 6.vii.55	27	♀	27
9 6.xii.55	28‡	?	7
	29	?	15
	30	♀	23

* Advanced intra-uterine specimen not included.

† Both skin and hair sampled from 48 days onward.

‡ Complete serial sections prepared.

of skin from each sample were sectioned serially at 8 μ , one parallel to the skin surface, and the other to the long axes of the hair follicles. All the sections were stained in haemalum, eosin, and picric acid. Some unstained whole mounts of skin were also prepared.

(d) *Terminology*

A new terminology for pelage hair follicles in *P. nasuta*, based on one proposed by Hardy and Lyne (1956a) for wool follicles in sheep, is used in preference to that adopted by Gibbs (1938) or Hardy (1947).

Primary follicles (*P*) and *secondary follicles* (*S*) are recognized. The first-formed follicles are termed *original central primary follicles* (*PCO*). A *PCO* follicle occupies the central position of a follicle group and it is readily distinguished during development by the presence of a simple sudoriferous gland. Two later-formed follicles which usually appear simultaneously on either side of the *PCO* follicle are termed *original lateral primary follicles* (*PLO*). A sudoriferous gland is sometimes, but not always, formed in association with a *PLO* follicle. After the formation of this trio group the *original secondary follicles* (*SO*) develop, initially between the *PCO* and *PLO* follicles on the ental side of the group. No sudoriferous glands are formed in association with the *SO* follicles. All original follicles arise directly from the epidermis and have independent orifices on the skin surface.

Before the end of the first hair growth cycle a new generation of follicles develops by branching from the *PLO* and *SO* follicles. Those which arise from the *PLO* follicles are termed *derived lateral primary follicles* (*PLD*) and those which arise from the *SO* follicles are termed *derived secondary follicles* (*SD*). During the second and later cycles of hair growth more *PLD* and *SD* follicles develop from the *PLO* or *PLD* follicles or both, and from the *SO* or *SD* follicles or both. Follicles which arise from the *PCO* follicles are termed *derived central primary follicles* (*PCD*). All these derived follicles arise by branching from existing follicles and share a common orifice with their parent.

The terms for the various components of the follicle group are similar to those used by Hardy and Lyne (1956c).

III. THE DEVELOPMENT OF INDIVIDUAL FOLLICLES

(a) *Stages of Follicle Development*

The eight fundamental stages in hair follicle development for mammals in general (Hardy and Lyne 1956b, 1956c) can be recognized in both *P* and *S* follicles in *P. nasuta*.

(i) *Original Central Primary Follicles*.—See Figure 1. The various stages are:

Stage F1: Follicle plug.—A plug of cells from the epidermis extends into the dermis. An aggregation of dermal cells appears beneath the epidermal plug (Plate 2, Fig. 1).

Stage F2: Pre-papilla.—The base of the epidermal plug flattens prior to invagination when it is still relatively short; the length of the plug does not usually exceed its diameter, and is sometimes considerably less (Plate 2, Fig. 2).

Stage F3: Papilla.—The base of the plug becomes recognizably concave and it can be subdivided according to the shape of the dermal papilla and the development of the associated glands. At stage *F3a* (Plate 2, Fig. 3) the base of the epidermal plug becomes recognizably concave, the length of the follicle being less than its diameter. At stage *F3b* (Plate 2, Fig. 4) the length of the follicle is usually slightly

more than its diameter and the sudoriferous gland rudiment appears as a solid plug from the ental side of the follicle. The length of the dermal papilla is less than its diameter. At stage *F3c* (Plate 2, Fig. 5) the length of the follicle is more than twice its greatest diameter and the length of the papilla is equal to or greater than its diameter. A bilobed sebaceous gland with differentiated cells is formed below the junction of the sudoriferous gland with the follicle. Further development of the sudoriferous gland has taken place and it may show a distinct swelling.

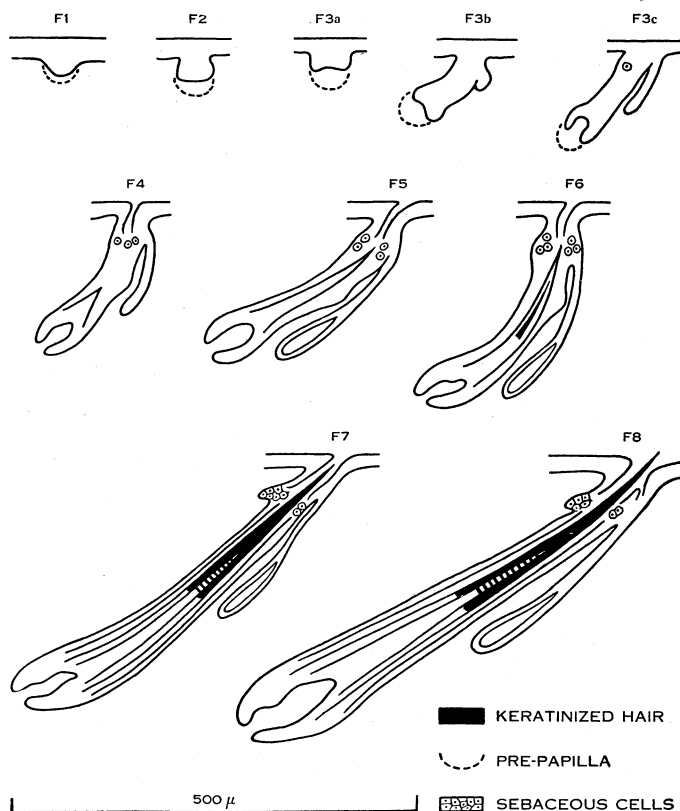


Fig. 1.—Diagram from camera lucida drawings of stages *F1*–*F8* in development of original central primary pelage hair follicles in *P. nasuta*.

Stage F4: Hair cone.—The hair cone is formed by the cells of Henle's layer of the inner root sheath (Plate 2, Fig. 6). The length of the follicle is approximately three to four times its greatest width. A hair canal containing keratinized cells is formed and it opens at the skin surface. An ental swelling of the outer root sheath is usually formed and the sudoriferous gland may contain a small lumen.

Stage F5: Advanced hair cone.—The tip of the hair cone is refractile and it reaches the level of the base of the sebaceous gland (Plate 2, Fig. 7). Inside the hair cone Huxley's layer and the developing hair cuticle and cortex can be recognized. Some follicles still have a distinct ental swelling of the outer root sheath.

Stage F6: Hair formation.—The tip of a keratinized hair appears inside the hair cone which is solid and refractile, and lies at or above the level at which the sebaceous gland opens. All the follicle layers can be recognized and a lumen is formed within every sudoriferous gland.

Stage F7: Hair in epidermis.—The tip of the hair has emerged through the inner root sheath layers and lies at the epidermal level in the hair canal on the ental side of the follicle (Plate 3, Fig. 1). The inner root sheath layers split and fragment as they reach the hair canal. No ental swelling of the outer root sheath can be recognized.

Stage F8: Hair emerged.—The tip of the hair has penetrated the superficial layers of the epidermis.

(ii) *Original Lateral Primary Follicles.*—Stages *F1* to *F3a* are similar to those of *PCO* follicles except that the follicle is usually more elongated at *F2* and *F3a*. A sudoriferous gland is not usually formed in association with a *PLO* follicle, but when it is formed, stage *F3b* is recognized. At stage *F3c* the rudiment of the sebaceous gland, with differentiated sebaceous cells, is formed; the length of the dermal papilla is equal to or greater than its diameter. Stages *F4* to *F8* are similar in most respects to those for *PCO* follicles. No ental swelling of the outer root sheath has been observed in association with any *PLO* follicles.

(iii) *Original Secondary Follicles.*—The *SO* follicles go through the same stages of development as the *PLO* follicles except that they are usually more elongated and a sudoriferous gland is not formed at any stage.

(iv) *Derived Lateral Primary and Derived Secondary Follicles.*—See Figure 2 and Plate 4. The *PLD* and *SD* follicles go through the same stages of development as the original ones, except that:

- (1) Stage *F1* is a budding from a *PLO*, *PLD*, *SO*, or *SD* follicle at a level immediately below that at which the sebaceous gland opens, and not from the epidermis.
- (2) No additional sudoriferous or sebaceous gland is formed.
- (3) The hair canal, or hair funnel, into which the *PLD* or *SD* hair enters at stage *F7* is the common canal for the follicle bundle which was formed during the development of the *PLO* or *SO* follicle.

(v) *Derived Central Primary Follicles.*—*PCD* follicles are uncommon, and as they do not arise until after the first *PLD* and *SD* follicles are formed, only limited observations on their development have been possible.

A unique feature of the branching of *PCO* follicles, and some *PLO* follicles, is that the type of hair which develops within the derived follicle is different in size and form from that which develops within the parent follicle (Fig. 7). All the *PCO* follicles, and some *PLO* follicles, develop only large grooved hairs, whereas the *PCD* and *PLD* follicles develop only small circular hairs. In all skin samples examined the branching of the *PCO* follicle is at a level immediately below that at which the sebaceous gland opens.

(b) *The Elongation of Original Central Primary Follicles*

As most of the first generation *PCO* follicles are straight or only slightly bent it has been possible to measure them at different stages of development. Measure-

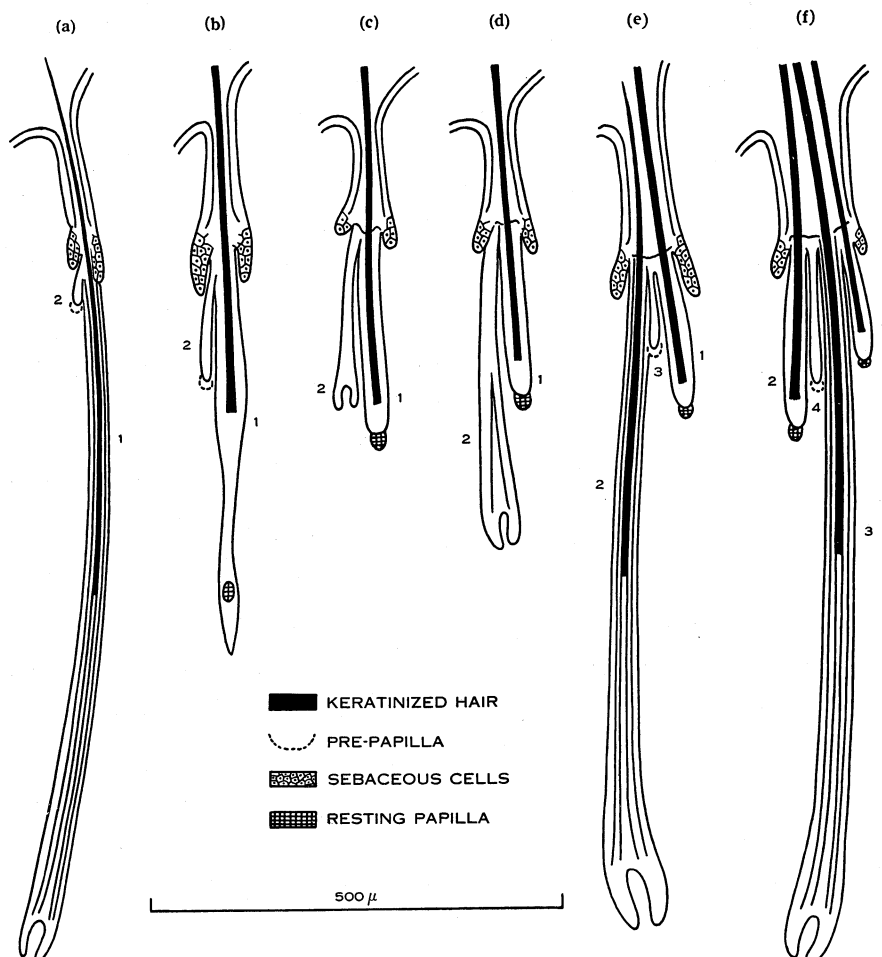


Fig. 2.—Diagram from camera lucida drawings of some of the stages in development of a bundle of lateral primary or secondary pelage hair follicles in *P. nasuta*. (a) Original follicle (1) at stage *F8* and first derived follicle (2) at stage *F1*. (b) Original follicle (1) with degenerating hair bulb, hair club, and resting papilla. First derived follicle (2) at stage *F1*. (c) Original follicle (1) with hair club and resting papilla. First derived follicle (2) at stage *F3c*. (d) First derived follicle (2) at stage *F4*. (e) First derived follicle (2) at stage *F8*, and second derived follicle (3) at stage *F1*. (f) First derived follicle (2) with hair club and resting papilla. Second derived follicle (3) beyond stage *F8* and third derived follicle (4) at stage *F1*. Follicle regeneration from the cells between the hair club and surviving cells of the papilla has not been observed.

ments of follicle length (i.e. the length along the long axis of the follicle, or in a straight line if the follicle is bent, from the epidermal-dermal junction to the base of the bulb) have been made with an ocular micrometer on serial sections cut parallel to the long axes of the follicles. The relation between the estimated age and the

length of the first generation *PCO* follicles up to 48 days is shown in Figure 3. Only the most advanced stage *F1* follicles have been measured.

The length of the follicle increases very rapidly after the emergence of the hair. At an estimated age of 40 days, when the most advanced *PCO* follicles are at stage *F8*, the follicle length is approximately 0.55 mm. Eight days later the *PCO* follicles have increased in length to about 2.0 mm, and the *PCO* hairs have grown

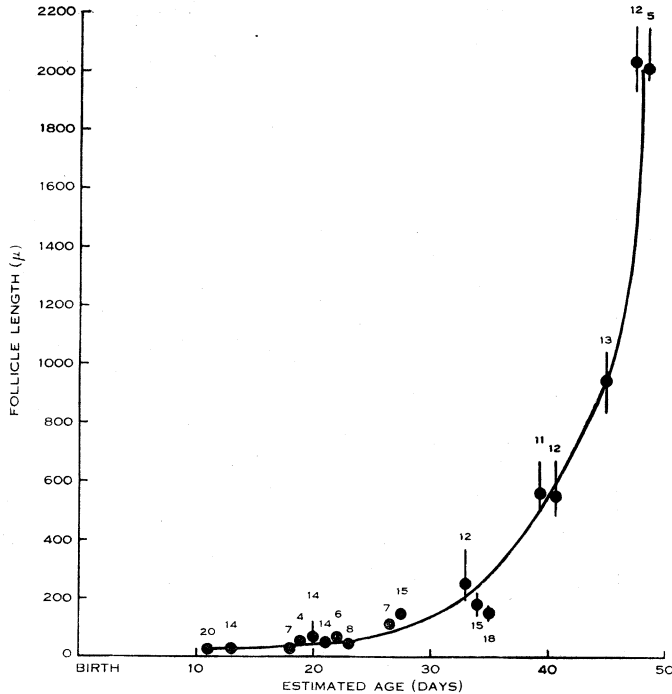


Fig. 3.—Relation between the estimated age and the length of the first generation original central primary follicles in *P. nasuta*. The number of follicles measured and the range of follicle length is shown when it falls outside the plotted mean. The curve through the points has been drawn by free-hand.

to a length of approximately 2.0 mm above the epidermis. No further elongation of the first generation *PCO* follicles has been observed although the hairs in these follicles increase to a final length of approximately 15.0 mm above the epidermis. The upper and lower parts of a fully developed *PCO* follicle at 48 days are illustrated in Plate 3, Figures 2, 3, and 4.

(c) Rate of Development of Follicles

The relation between the estimated age and the most advanced follicle stage found among the different types of follicles of the first hair cycle is shown in Figure 4. The *PCO* follicles begin their development on the midside between the 7th and 11th day after birth and reach stage *F8* in 29–33 days. The *PLO* follicles begin 23–27 days after birth and reach *F8* in 18–22 days, and the *SO* follicles, which begin at 27–34 days, reach *F8* in 14–21 days.

The first derived follicles begin their development while the follicles of the first hair cycle are still active. A few *PLD* follicles have been observed at 45 days, and at 48 days most of the *PLO* and *SO* follicles have derived follicles associated with them (Plate 5, Fig. 4). These derived follicles are at first relatively very small; they remain slender and appear to remain stationary at stage *F2* as long as their parent follicles are still actively growing. At 54, 61, and 62 days the most advanced derived follicles are still at stage *F2*. At 75 days the most advanced derived follicles

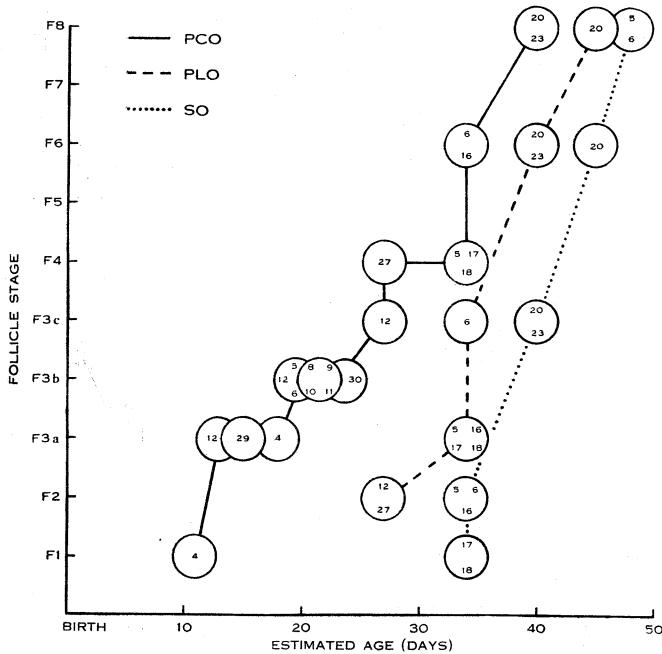


Fig. 4.—Relation between the estimated age and the most advanced follicle stage found among the different types of follicles of the first hair cycle in *P. nasuta*. The specimen numbers (see Table 1) are shown in the circles.

are at *F4*, and at 76 days one specimen has derived follicles at *F6* and its litter mate has derived follicles at *F8*. From these observations it was estimated that the first *PLD* and *SD* follicles developed from stage *F1* to stage *F8* in approximately 28–31 days. However, if the stationary phase is omitted, the rate of development of the first derived follicles is similar to that for the first *SO* follicles.

The first *PCD* follicle discovered was at an early stage of development in a specimen (No. 5) at 83 days. The same specimen at 97 days has some *PCD* follicles beyond stage *F8* (Fig. 7).

(d) *The Relation between the Follicle Types in the Skin and the Hair Types in the Coat*

Two main types of hair are readily distinguished in the coat of *P. nasuta*. The *PCO* follicles always produce coarse flattened guard hairs (Figs. 5(f), 5(g), and 5(i);

Plate 5, Fig. 4) which are grooved on the ectal side along the greater part of the shaft. These characteristic guard hairs are similar to the protective hairs described by Lyne and McMahon (1951) in the bandicoots *I. obesulus* and *P. gunnii*. The *S*

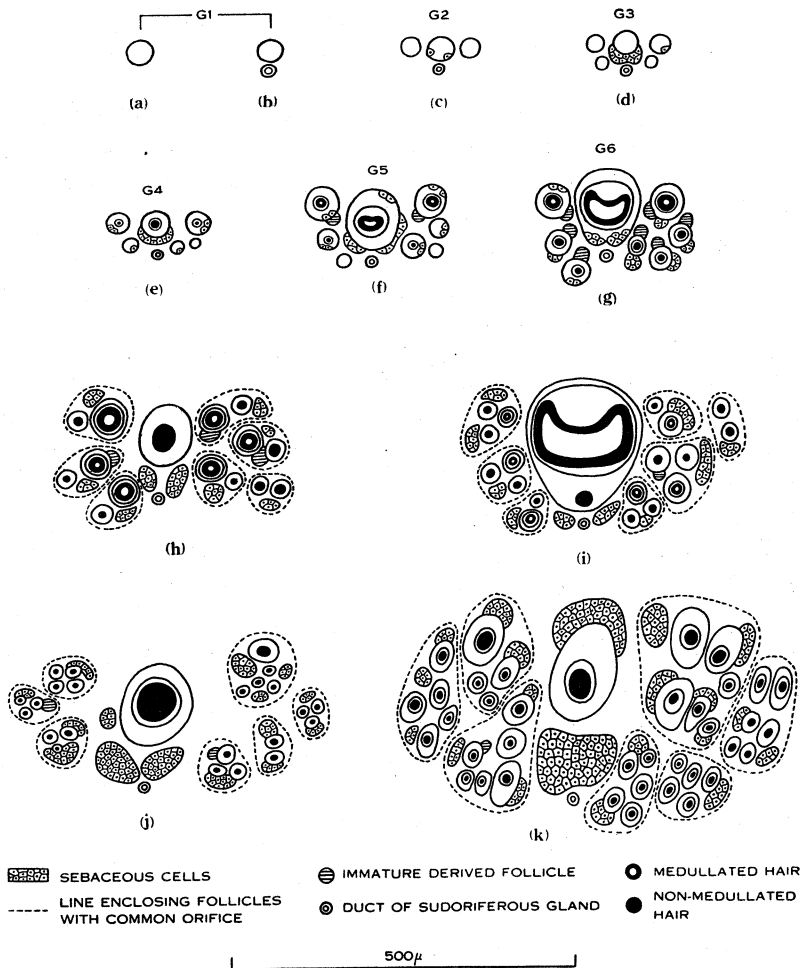


Fig. 5.—Diagram from camera lucida drawings of stages in development of the follicle group in *P. nasuta*. The sections are transverse to the follicles at the level of the sebaceous glands. (a)–(g) Stages G1–G6 of the first hair cycle. Stage G1 begins when the first original central primary follicles are at stage F1 at about 11 days after birth. Stage G6 is reached when the first original secondary follicles have emerging hairs at about 48 days of age. (h)–(k) The follicle group at approximately 83, 97, 125, and 377 days after birth. Dotted lines enclose the bundles of closely associated lateral primary and secondary follicles which have a common orifice.

follicles always produce fur hairs which are fine and circular in cross section. Some grooved hairs, usually smaller than those produced by the PCO follicles, are produced by the PLO follicles. All the hairs are medullated, except at the tip and base.

IV. THE DEVELOPMENT OF FOLLICLE GROUPS

(a) *Stages in the Development of the Follicle Group of the First Hair Cycle*

The main events in the development of the follicle group of the first hair cycle (Figs. 5(a)–5(g); Plate 5, Figs. 1, 2, 3, and 4) may be described by a series of stages prefixed by the letter *G* to distinguish them from the *F* stages of individual follicles. The stage numbers do *not* correspond with those proposed by Carter (1943) for the Merino and redefined by Hardy and Lyne (1956c).

Stage G1: PCO follicles present.—These follicles, which are more or less equally spaced on the skin surface, do not all develop at the same time. It is convenient to subdivide this stage. At stage *G1a* (Fig. 5(a)) the follicles are at stage *F1* to *F3a*. At stage *G1b* (Fig. 5(b)) the most advanced follicles are at stage *F3b*.

Stage G2: Trio groups formed by the addition of a PLO follicle on each side of some of the PCO follicles (Fig. 5(c)).—The most advanced *PCO* follicles are at stage *F3c*.

Stage G3: SO follicle formation begun (Fig. 5(d)).—The first plugs (*F1*) of *SO* follicles appear between the *PCO* and *PLO* follicles on the ental side of the group (Plate 5, Fig. 3). *PCO* follicles are at stage *F4* and *PLO* follicles are at *F3a*.

Stage G4: PCO hairs emerged (Fig. 5(e)).—Some *PCO* follicles are at *F8*. Some *PLO* follicles are at *F6* and some *SO* follicles are at *F3c*.

Stage G5: PLO hairs emerged (Fig. 5(f)).—Some *PLO* follicles are at *F8* and some *SO* follicles are at *F6*.

Stage G6: SO hairs emerged (Fig. 5(g)).—Some *SO* follicles are at *F8*.

(b) *The Development of the Follicle Group of the Second and Later Hair Cycles*

At stage *G5* the first *PLD* follicles appear immediately below the level at which the sebaceous gland opens, usually on the side of the *PLO* follicle nearest to the *PCO* follicle (Fig. 5(f)). At stage *G6* the first *SD* follicles appear at the same level as the *PLD* follicles, usually on the side of the *SO* follicle nearest to the *PCO* follicle (Fig. 5(g); Plate 5, Fig. 4). At this stage (*G6*) the first generation *PCO* follicles and most of the *PLO* follicles cease to increase in length. The most advanced *PLD* and *SD* follicles are at *F2*, and appear to remain stationary until the growth of the follicles of the first hair cycle ceases, and hair clubs are being formed. The hair canal into which the *PLD* or *SD* hair enters at stage *F7* is the common canal for the follicle bundle which was formed during the development of the *PLO* or *SO* follicle. When the first *PLD* and *SD* follicles reach stage *F8*, most of the *PLO* and *SO* hairs are still present as hair clubs (Figs. 2(e) and 2(f)).

Only an occasional *PCO* follicle gives rise to *PCD* follicles. There is, however, a normal replacement of *PCO* hairs as shown in Figure 6, the new hairs developing on the ectal, or concave side, of the old hairs.

Before the end of the second and later hair cycles, more *PLD* and *SD* follicles arise by branching from the *PLO* or *PLD* follicles or both and from the *SO* or *SD* follicles or both (Figs. 2(e) and 2(f)). When the second generation of derived follicles reaches stage *F8* the follicle bundle is usually composed of three closely associated follicles sharing a common orifice.

(c) *The Size of the Follicle Group*

Observations on the size of the follicle group at different ages have been made on the serial sections cut parallel to the skin surface. Counts have been made of the number of: original follicles in each group during the first hair cycle, orifices per

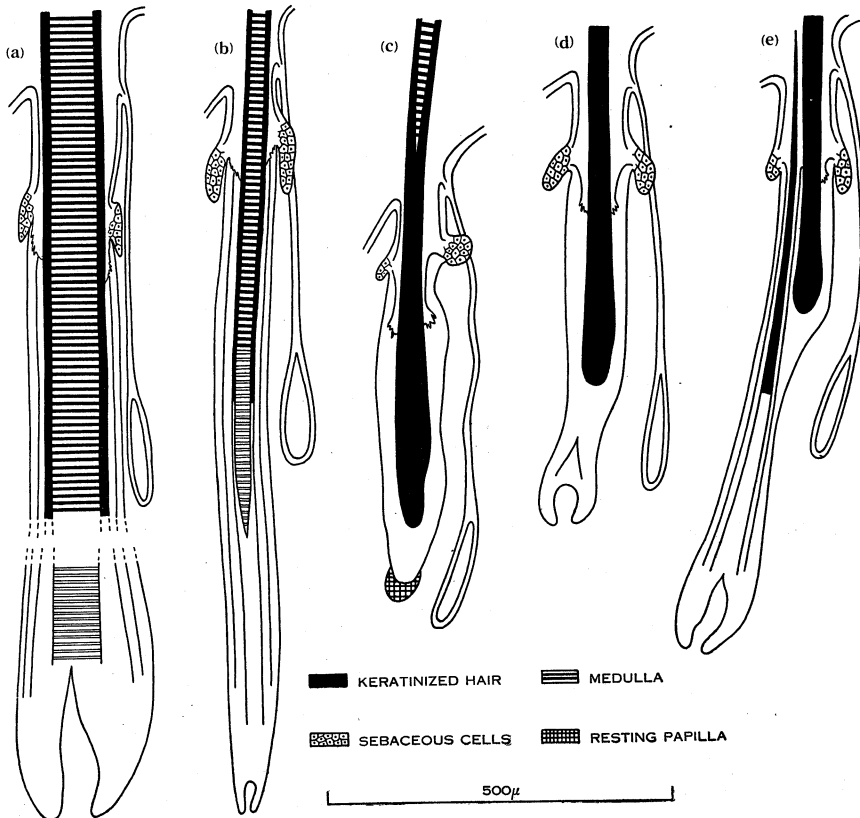


Fig. 6.—Diagram from camera lucida drawings of some of the changes in the original central primary follicles during a hair growth cycle in *P. nasuta*. (a) Upper and lower parts of a fully developed follicle. The follicle is at its maximum length of approximately 2.0 mm. (b) Beginning of resting phase. The hair has become thin at the base and its growth has almost ceased. A non-medullated hair club is being formed and the follicle bulb has decreased in size. (c) Resting phase. The follicle has shortened and a hair club is formed. The former dermal papilla cells are clustered at the base of the epidermal sheath surrounding the hair club. (d) A new follicle root, formed by proliferation of cells at the base of the resting follicle, has reached a stage equivalent to stage *F4* of the first hair cycle. (e) The new hair has emerged on the ectal (grooved) side of the old hair.

group, hairs per group, and hairs per orifice in the second and subsequent cycles. Summaries of the observations on the size of the follicle group from 34 to 45 days, and from 48 to 526 days are shown in Tables 2 and 3 respectively. These periods cover most of the first hair cycle, and the second and subsequent cycles respectively.

The counts of the number of original follicles per group (Table 2) reveal that in three out of the five specimens at 34 days, some of the groups contain only two

follicles; that is, some of the *PLO* and *SO* follicles have not appeared. Also, the mean number of follicles per group is lower for these three specimens (Nos. 16, 17, 18; litter mates) than it is for the other specimens (Nos. 5, 6; also litter mates) at the

TABLE 2
SUMMARY OF OBSERVATIONS ON THE SIZE OF THE FOLLICLE GROUP FROM 34 TO 45 DAYS

Estimated Age (days)	Specimen No.	No. of Follicle Groups Examined	No. of Original Follicles per Group	
			Range	Mean
34	5	55	3-14	6.6
34	6	29	3-9	5.7
34	16	37	2-9	4.5
34	17	28	2-6	3.9
34	18	30	2-7	3.6
40	20	39	3-14	6.9
40	23	42	4-13	8.0
45	20	33	3-8	5.4

same estimated age. Although one specimen (No. 5) at 34 days has a few larger follicle groups and a higher mean number of original follicles per group than its

TABLE 3
SUMMARY OF OBSERVATIONS ON THE SIZE OF THE FOLLICLE GROUP FROM 48 TO 526 DAYS

Estim- ated Age (days)	Speci- men No.	No. of Follicle Groups Exam- ined	No. of Orifices per Group*		Total No. of Hairs per Group		No. of <i>PC</i> Hairs per Orifice		No. of <i>PL</i> and <i>S</i> Hairs per Orifice	
			Range	Mean	Range	Mean	Range	Mean	Range	Mean
48	5	94	4-11	6.3	4-11	6.3	0	1.0	0	1.0
48	6	26	4-11	6.9	4-11	6.9	0	1.0	0	1.0
97	5	21	3-10	5.7	7-30	15.9	1-4	2.1	0-6	3.0
110	22	14	4-8	6.4	9-23	15.3	0	2.0	1-4	2.5
125	5	32	3-10	5.9	8-27	15.0	1-4	1.8	0-6	2.7
153	6	14	4-9	5.9	15-40	22.9	1-4	1.4	1-8	4.4
166	22	10	3-7	4.8	11-27	16.9	1-2	1.1	2-7	4.2
222	22	16	3-9	5.8	8-27	16.4	1-3	1.7	1-6	3.1
265	6	19	3-7	4.3	7-37	19.8	1-6	2.4	1-8	5.3
377	6	7	4-9	6.6	19-49	34.1	1-3	1.6	0-11	5.8
463	22	10	4-11	6.5	14-33	22.0	1-2	1.4	1-7	3.8
526	22	27	3-9	6.0	13-36	24.1	1-3	1.3	1-10	4.6

* Equals the number of original follicles per group.

litter mate (No. 6), the most advanced *PCO* and *PLO* follicles in the latter specimen are at a later stage of development than the same follicle types in the former specimen (Fig. 4). At 40 days both specimens (litter mates) have several large follicle groups

and the mean number of original follicles per group is especially large in one specimen. If the number of original follicles per group from 34 to 45 days (Table 2) is compared with the number of orifices per group from 48 to 526 days (Table 3), it is found that, with the exception of three specimens (Nos. 16, 17, 18) at 34 days, there are very

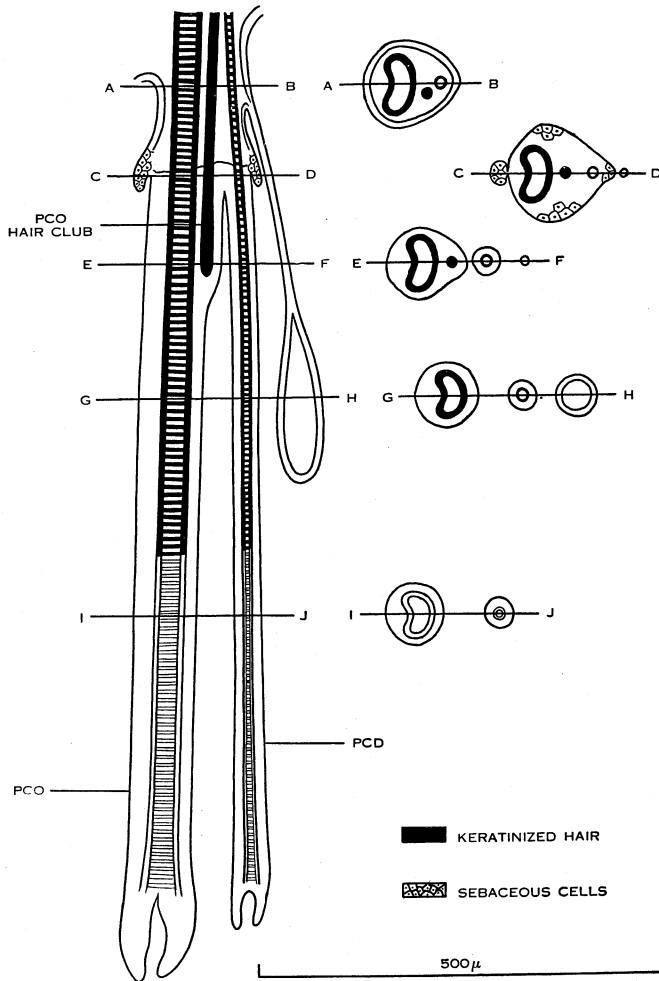


Fig. 7.—Diagram from camera lucida drawings of a bundle of central primary pelage hair follicles in *P. nasuta*. Specimen 97 days old. Longitudinal section on left is drawn from reconstruction of transverse sections, several of which are shown on the right. The first generation *PCO* hair is still present as a hair club. Note that the *PCD* hair differs in size and form from the new *PCO* hair.

few differences between the two sets of figures. These counts confirm other observations that only the follicles of the first hair cycle arise from the epidermis. The number of orifices per group, which equals the number of original follicles per group, remains constant after all the follicles of the first hair cycle have been formed at about 40 days.

Three specimens, one at 34 days and two at 40 days, have a few groups containing up to 13 or 14 original follicles (Table 2). In contrast, the number of orifices per group from 48 to 526 days does not exceed 11 (Table 3). This suggests that the larger groups at 34 and 40 days, which usually have a sudoriferous gland associated with one or even both *PLO* follicles, later separate into two (possibly three) distinct groups. If this occurs it is thought that a *PLO* follicle becomes the central member of the new follicle group. This *PLO* follicle would then be classified as a *PCO* follicle. It is possible that the few *PCO* follicles which branch (Fig. 7) were initially *PLO* follicles but, with the present material, there does not seem to be any way of testing this hypothesis.

With only one exception, not more than two *PCD* follicles in a *PC* bundle, and not more than four *PC* hairs per orifice, have been observed. The exceptional *PC* orifice has six hairs (Table 3) but no sudoriferous gland is discernible. This is further evidence that a few *PLO* follicles later occupy the central position of a group.

The figures for the number of *PL* and *S* hairs per orifice (Table 3) show that, from 97 to 526 days, bundles with 2, 3, 4, or 5 hairs are common. The largest bundle examined contains 11 follicles with emerging hairs.

(d) *The Density of the Follicles*

Only very limited studies have been made on the density of the hair follicles because the skin samples collected were of various sizes and no corrections were made for changes in area at collection or during histological preparation.

Counts were made of the *PC* and *PL* plus *S* follicles (immature derived follicles not included) occurring in sections of skin of areas of 1–4 mm², cut parallel to the surface and at the sebaceous gland level. Two specimens (Nos. 5, 22) were examined at estimated ages ranging from 48 to 526 days. At 48, 97, 125, 166, 222, and 526 days the approximate number of *PC* follicles per mm² was 26, 21, 17, 11, 13, and 8 respectively, and, in the same specimens at the same ages, the approximate number of *PL* plus *S* follicles per mm² was 130, 152, 161, 163, 107, and 140 respectively. As the number of original follicles is complete before 48 days, and there is little branching from the *PCO* follicles, it seems probable that the changes in density of the *PC* follicles merely reflect the normal skin expansion accompanying growth.

V. DISCUSSION

Histological study of the development and replacement of pelage hairs in *P. nasuta* has revealed several unique features not previously described in any mammal.

In the development of the *PCO* follicles of *P. nasuta* the pre-papilla stage (*F2*) and the first invagination (*F3a*) occur when the follicle is still relatively very short and before the first appearance of the sudoriferous gland rudiment. In *T. vulpecula*, Gibbs (1938) figures the formation of a sudoriferous gland rudiment prior to the invagination of the base of the follicle. In the central and lateral primary follicles of sheep, described by Hardy and Lyne (1956c), the sudoriferous gland rudiment appears at stage *F2*, and the follicle is considerably more elongated than it is in *P. nasuta* at stage *F3a*.

In *P. nasuta*, a sudoriferous gland may develop in association with one or both *PLO* follicles but a typical group has only one sudoriferous gland associated with the *PCO* follicle. Hardy (1947) described sudoriferous glands associated with lateral primary follicles in one of three specimens of *P. nasuta* which she examined. In the various breeds of sheep examined by Carter (1955) a sudoriferous gland was always formed in association with the lateral primary as well as the central primary follicles.

The *SO* follicles in *P. nasuta*, which develop on the ental side of the group, may be compared with the *SO* follicles in sheep (Hardy and Lyne 1956c). In sheep, the first-formed *SO* follicles develop on the ectal side of the group.

The *PCO* follicles in *P. nasuta* develop from stage *F1* to stage *F8* in about 29–33 days while the equivalent follicles in the mouse (Hardy 1949) and the sheep (Hardy and Lyne 1956c) pass through the same stages in about 9 and 38 days respectively. In *P. nasuta*, the *PLO* and *SO* follicles, particularly the latter, develop at a faster rate than the equivalent follicles in the sheep. The *SO* follicles in *P. nasuta* develop from *F1* to *F8* in about 14–21 days whereas the *SO* follicles in the sheep pass through the same stages in about 40 days. This suggests that the branching of the *SO* follicles, which begins at a much earlier stage in the sheep than in *P. nasuta*, has considerable influence on the rate of development of these follicles.

The replacement of the *PCO* follicles in *P. nasuta* is similar in most respects to that which has been described by Segall (1918) for the guinea pig, and by Dry (1926) for the mouse. The method of replacement of the *PLO* and *SO* follicles in *P. nasuta* apparently has not been previously described in mammals. There is, however, some evidence to suggest that it may be a common one. In the marsupial mole, *Notoryctes typhlops*, the hairs are arranged in bundles usually containing from 11 to 19 hairs in separate root sheaths (Sweet 1907). The follicles of each bundle have a common neck and a common orifice at the skin surface. The development of these bundles was not described but it seems probable that follicle branching occurs in this marsupial. In the adult marsupial material examined by Hardy (1947) the typical follicle group consisted of one large central hair follicle flanked by from two to eight lateral bundles of smaller follicles, each bundle with a common follicle opening at the skin surface. Branching follicles are not described by Hardy (1947) but in her Plate V, Figure 12, illustrating a follicle group from the bandicoot, *Isoodon torosus* Ramsay, she shows several immature follicles which are probably derived follicles similar to those described in the present study.

In the branching secondary wool follicles of sheep, described by Hardy and Lyne (1956c), the branching is usually at or above the level of the sebaceous glands and it begins at a much earlier stage in the development of the *SO* follicles than in *P. nasuta*. Also, the type of follicle branching in sheep is associated with the maturation of the follicle group and not with the replacement of hair as it is in *P. nasuta*. No branching primary follicles have been described in sheep.

According to Weddell and Pallie (1955) as many as seven separate hairs, derived from closely related hair follicles, may emerge from a single small orifice in the skin of the rabbit ear. The development of these bundles of follicles is not described but the observations suggest that follicle branching occurs in this mammal.

In the adult chinchilla Wilcox (1950) described bundles with as many as 75 hairs emerging from a single orifice; this would suggest that branching occurs in this species. In the mouse, Dry (1926) says that bundles of two, three, or four hairs are grown, one by one, in the same follicle. However, he describes two exceptional bundles in the tail of an animal more than a year old; each bundle had two hairs in separate root sheaths below the persistent part of the follicle. In the platypus (*Ornithorhynchus*) the adult follicle group consists of a central follicle surrounded by a number of bundles of follicles (Poulton 1894); each bundle was originally a simple follicle from which one fibre projected. Poulton says that the bundles of follicles are formed by branching from some part of the first-formed follicles, and sebaceous glands are situated at the level at which the follicles unite to form a common follicular neck. Spencer and Sweet (1899) examined hair development in both the spiny ant-eater (*Tachyglossus*) and the platypus and they confirmed Poulton's observations on the branching of follicles in the latter.

VI. ACKNOWLEDGMENTS

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EXPLANATION OF PLATES 1–5

All figures (except Plate 1) are of sections through skin from the mid-lateral region of the trunk of the bandicoot, *P. nasuta*. The ages of all specimens are estimated. The staining is with haemalum, eosin, and picric acid. *C*, stratum corneum; *Co*, cortex; *Cu*, cuticle of hair; *D*, derived lateral primary or derived secondary follicle; *DP*, dermal papilla; *ES*, ental swelling of outer root sheath; *G*, stratum germinativum; *H*, keratinized hair; *HC*, hair canal; *HF*, hair funnel; *HN*, hair cone; *M*, medulla; *O*, original lateral primary or original secondary follicle; *P*, pigment; *PCO*, original central primary follicle; *PLD*, derived lateral primary follicle; *PLO*, original lateral primary follicle; *PP*, pre-papilla; *RP*, resting papilla; *S*, stratum spinosum; *SCS*, space formed by disintegration of sebaceous cells; *SD*, derived secondary follicle; *SG*, sebaceous gland with differentiated cells; *SO*, original secondary follicle; *SudG*, sudoriferous gland; *SudGD*, sudoriferous gland duct; *SudGR*, sudoriferous gland rudiment.

PLATE 1

Perameles nasuta

- Fig. 1.—Specimen No. 5 at 34 days. Note that the specimen is naked except for vibrissae. The first hairs emerge on the mid-lateral region of the trunk at 40 days.
- Fig. 2.—The same specimen as shown in Plate 1, Figure 1, at 48 days. Note that the specimen is now completely covered with hair.
- Fig. 3.—Specimen No. 6 (litter mate of No. 5) at 321 days. The specimen is now almost fully grown.

PLATE 2

Longitudinal sections of original central primary follicles at stages *F1*–*F5*

- Fig. 1.—Two *PCO* follicles at stage *F1* in an 11-day-old specimen.
- Fig. 2.—Stage *F2* at 21 days.
- Fig. 3.—Stage *F3a* at 18 days.
- Fig. 4.—Stage *F3b* at 21 days.
- Fig. 5.—Stage *F3c* at 27 days.
- Fig. 6.—Stage *F4* at 34 days.
- Fig. 7.—Stage *F5* at 34 days. The tip of the hair cone is not clearly seen.

PLATE 3

Development of original central primary follicles

- Fig. 1.—Longitudinal section of a *PCO* follicle at stage *F7* in a specimen at 40 days.
- Fig. 2.—Longitudinal section of upper part of a fully developed *PCO* follicle in a specimen at 48 days.
- Fig. 3.—Longitudinal section of lower part of follicle shown in Plate 3, Figure 2.
- Fig. 4.—Almost transverse section of a fully developed *PCO* follicle at the level of the dermal papilla in a specimen at 48 days. The dermal papilla is grooved on the ectal side.

PLATE 4

Longitudinal sections of bundles of lateral primary or secondary follicles showing some stages of development

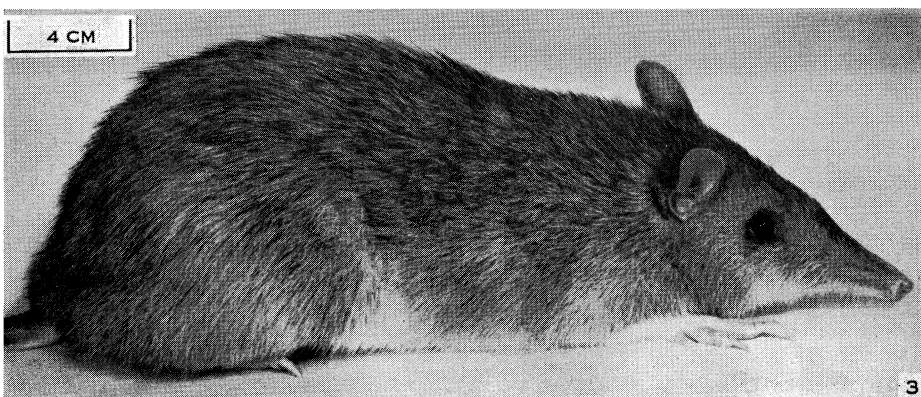
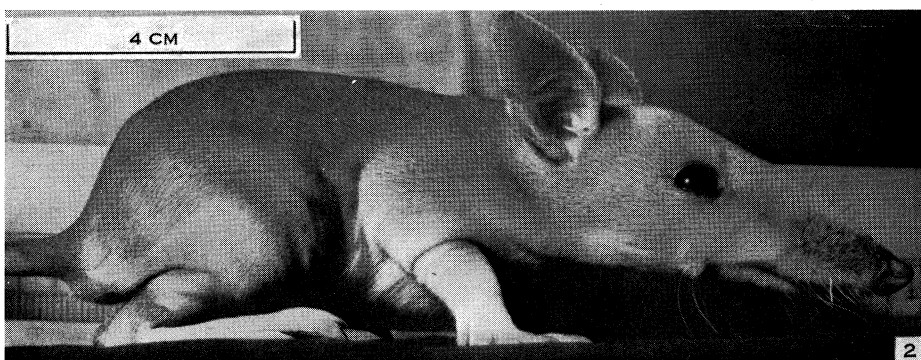
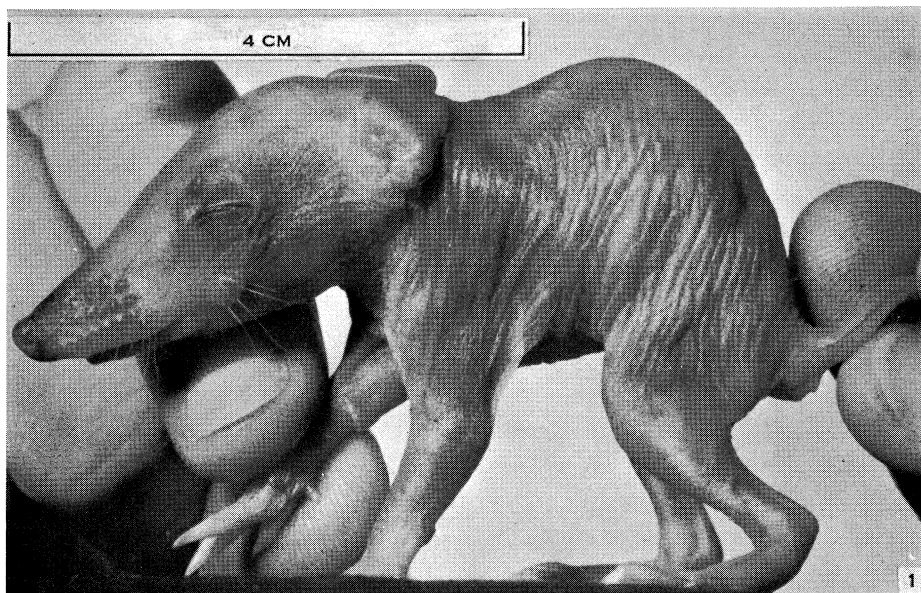
- Fig. 1.—Upper part of *SO* follicle at stage *F8* and *SD* follicle at stage *F1* in a specimen at 48 days.
- Fig. 2.—Part of *PLO* or *SO* follicle with hair club (not clearly seen) and derived follicle (*PLD* or *SD*) at stage *F1* in a specimen at 61 days.
- Fig. 3.—*PLO* or *SO* follicle with hair club and derived follicle at about stage *F3c* in a specimen at 75 days.
- Fig. 4.—New derived follicle with tip of pigmented hair at base of hair funnel and earlier formed follicle with hair club. Specimen at 153 days.
- Fig. 5.—Part of a bundle of three *PL* or *S* follicles in a specimen at 265 days. Active follicle in centre and resting follicles with hair clubs on either side.

PLATE 5

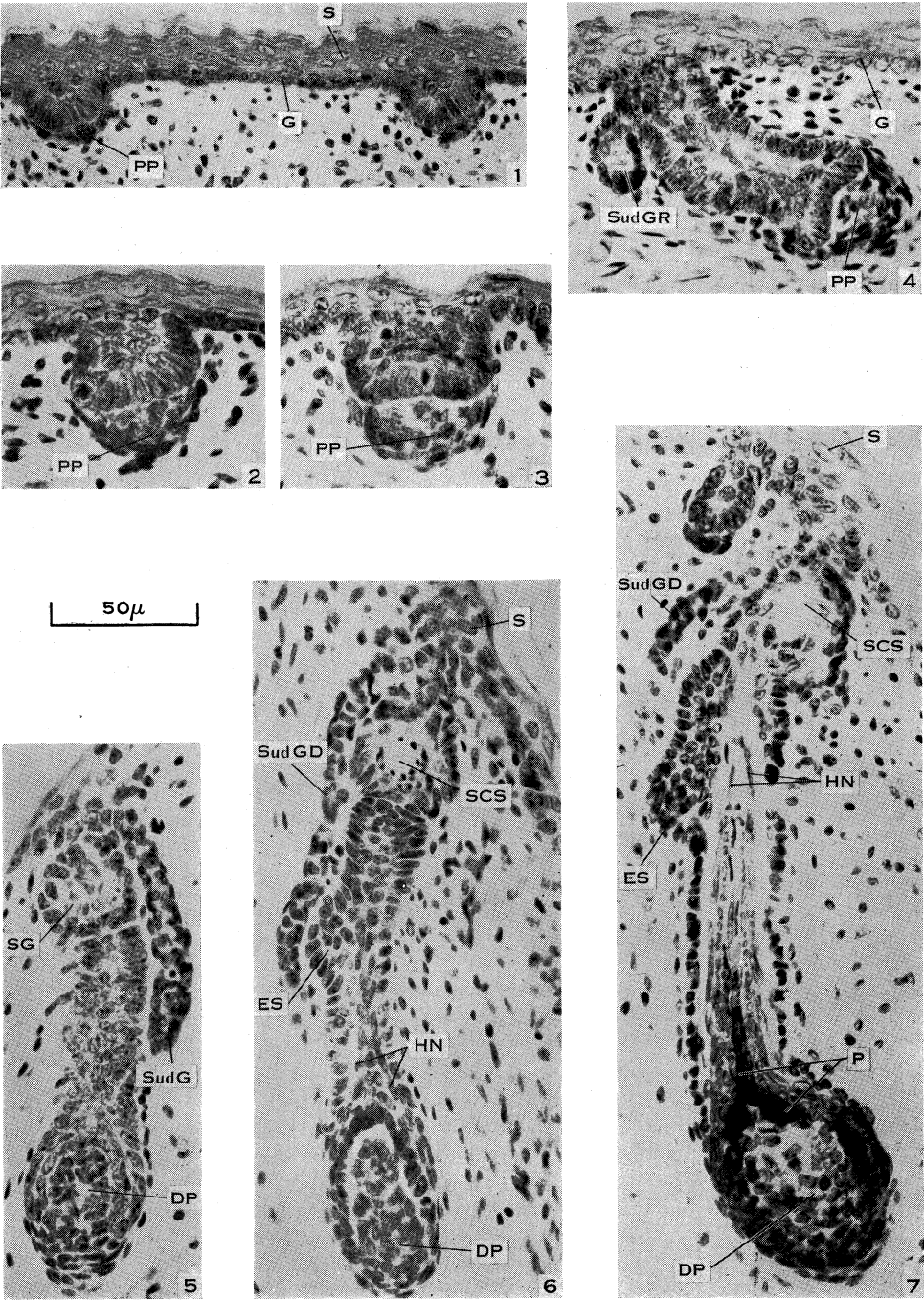
Transverse sections of follicle groups showing some stages of development

- Fig. 1.—Stage *G1a* at 20 days. *PCO* follicle at stage *F3a*.
- Fig. 2.—Stage *G2* at 27 days. *PCO* follicle at stage *F3b* and *PLO* follicles at stage *F2*.
- Fig. 3.—Stage *G3* at 34 days. *PCO* follicle at stage *F4*, *PLO* follicles at stage *F3a*, and *SO* follicles at stage *F1*.
- Fig. 4.—Stage *G6* at 48 days. *PCO*, *PLO*, and *SO* follicles at or beyond stage *F8*. *PLD* and *SD* follicles at *F1–F2*.
- Fig. 5.—Portion of a follicle group showing the *PCO* follicle and three complete bundles of follicles (one *PL* and two *S*) at a level between the epidermis and the sebaceous glands. Specimen 125 days old.
- Fig. 6.—Portion of follicle group shown in Plate 5, Figure 5, at a level below the sebaceous glands.
- Fig. 7.—A complete follicle group below the level at which the sebaceous glands open. Specimen 377 days old.

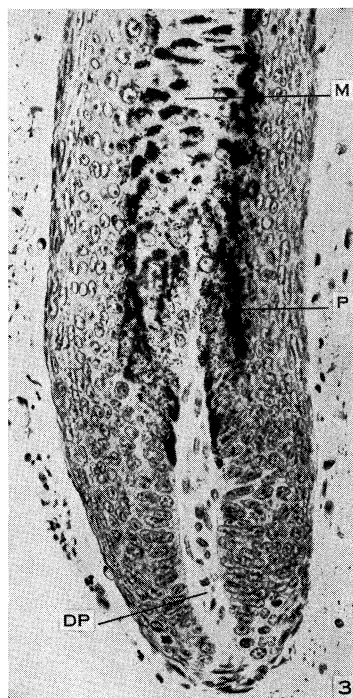
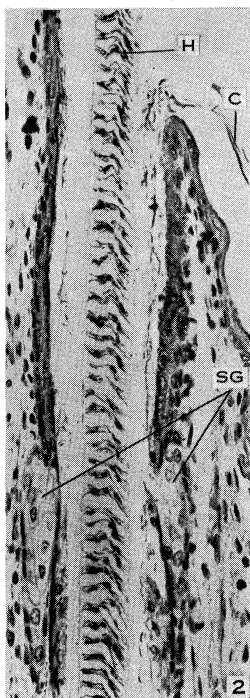
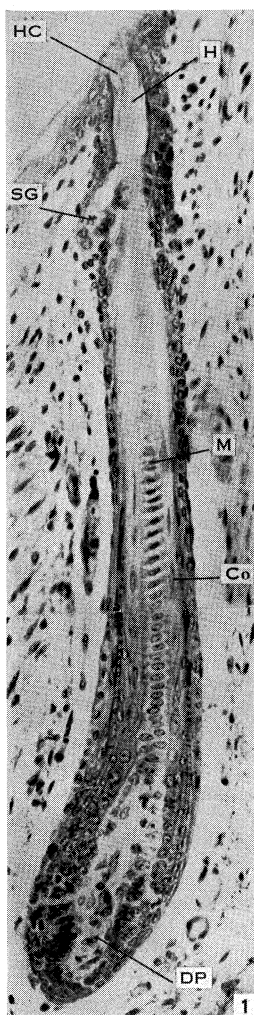
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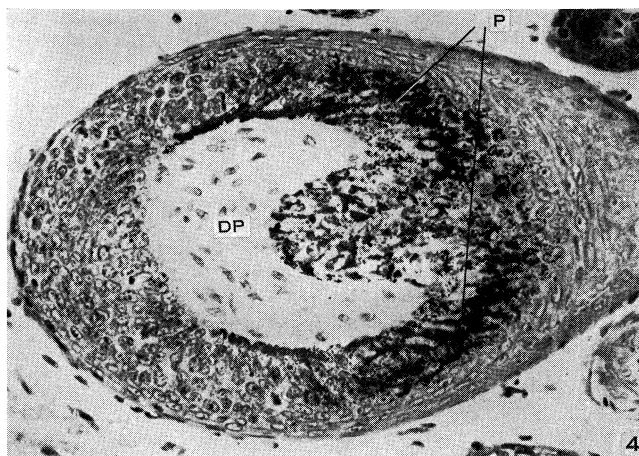
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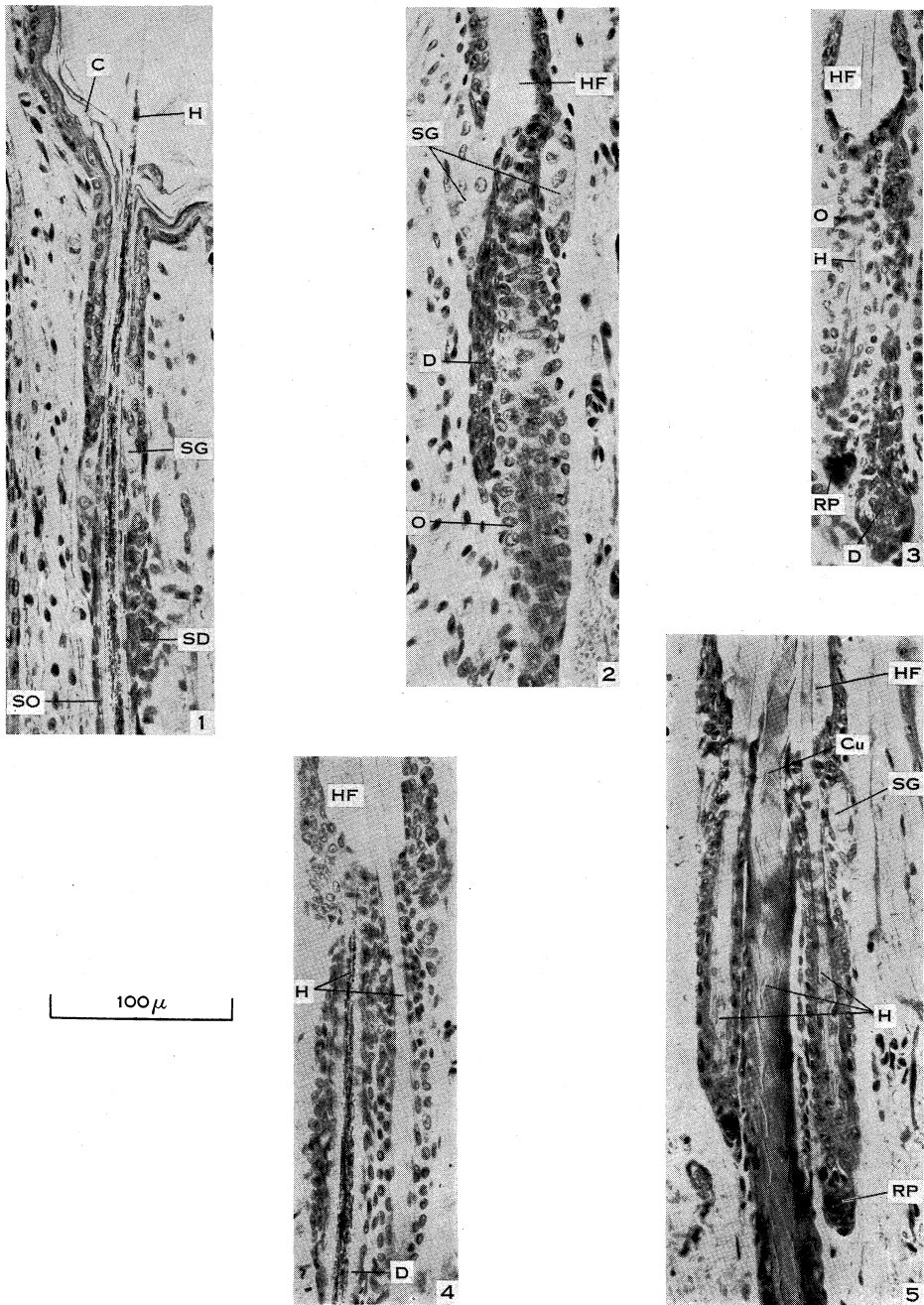
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